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Synthesis of isoquinuclidine analogs of chloroquine: Antimalarial and antileishmanial activity

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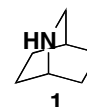
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Abstract—The isoquinuclidine (2-azabicyclo[2.2.2]octane) ring system may be viewed as a semi-rigid boat form of the piperidine ring and, when properly substituted, a scaffold for rigid analogs of biologically active ethanolamines and propanolamines. It is present in natural products (such as ibogaine and dioscorine) that display interesting pharmacological properties. In this study, we have expanded our continuing efforts to incorporate this ring system in numerous pharmacophores, by designing and synthesizing semi-rigid analogs of the antimalarial drug chloroquine. The analogs were tested in vitro against *Plasmodium falciparum* strains and *Leishmania donovani* promastigote cultures. Compounds **6** and **13** displayed potent antimalarial activity against both chloroquine-susceptible D6 and the -resistant W2 strains of *P. falciparum*. All analogs also demonstrated significant antileishmanial activity with compounds **6** and **13** again being the most potent. The fact that these compounds are active against both chloroquine-resistant and chloroquine-sensitive strains as well as leishmanial cells makes them promising candidates for drug development.
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1. Introduction

The isoquinuclidine (2-azabicyclo[2.2.2]octane) ring system (**1**) is present in natural products possessing interesting pharmacological properties such as the alkaloids of *Dioscorea hispida*, typified by dioscorine (**2**), mearsine (**3**), a minor alkaloid of the elaeocarpaceous plant *Peripentadenia mearsii*¹ that grows in the rain forests of north Queensland, and the iboga alkaloids, of which ibogaine (**4**) is the prototype. Dioscorine has been shown to be a toxic central nervous system depressant^{2,3} and a modulator of the nicotinic acetylcholine receptor.⁴

The most important members of these three groups of alkaloids are the iboga alkaloids.



Ibogaine (**4**, NIH 10567, EndabuseTM), a natural alkaloid found in the root, rootbark, stem, and leaves of the African shrub *Tabernanthe iboga*, was isolated over 100 years ago.^{5–7} Its structure was established 50 years later⁸ and 10 years later its first total synthesis was reported by Buchi.⁹ A number of isoquinuclidine-based pharmacophores prepared over the years have shown interesting pharmacological activities. Several reviews of the anti-addictive properties of analogs of the iboga alkaloids have appeared^{10–12} as well as a review of iboga alkaloids and their role as precursors of anti-neoplastic bisindole *Catharanthus* alkaloids.¹³ Recently the synthesis and biological evaluation of 18-methoxycoronaridine congeners as potential anti-addictive agents have been reported.¹⁴ More recently, the chemical and biological properties of isoquinuclidine (**4**) analogs have also been reviewed.¹⁵

Keywords: Antimalarial; Antileishmanial; Isoquinuclidine; Chloroquine.

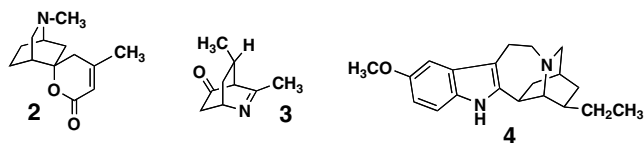
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Isoquinuclidine is basic, having a pK_a of 11, and may be considered as rigid analog of biologically active ethanolamines and propanolamines. This bicyclic amine thus provides structural similarity with the alkylamino side chain of several clinically useful drugs. We have recently expanded our continuing efforts of utilizing this ring system in a variety of pharmacophores to the synthesis of semirigid isoquinuclidine analogs of the widely used antimalarial chloroquine (**6**, **7**) (Fig. 1). Unfortunately, resistance to chloroquine has developed thus leading to its ineffectiveness¹⁶ and prompting the need for the development of new antimalarials.

2. Chemistry

Synthetic routes to the isoquinuclidine ring system have been reviewed recently by Borne and co-workers.¹⁵ Use of (+)-pulegone, in a Mannich-type reaction with acetaldehyde and benzylamine, produces an isoquinuclidine ring, which is a precursor to mearsine (**3**).¹⁷ Also 2-cyclohexenones with formaldehyde and methylamine produce 6-keto-isoquinuclidine ring systems, as in the synthesis of dioscorine (**2**).² Methylene-bis-urethane, prepared by Lewis acid-catalyzed Mannich reaction of urethane with formaldehyde, undergoes cycloaddition with 1,3-butadiene to form isoquinuclidine carbamate.¹⁸ Another widely used approach is via the epoxidation of the commercially available methyl 3-cyclohexene-1-carboxylate using 3-chloroperoxybenzoic acid (*m*-CPBA), the product of which subsequently reacts with amines

to yield an amino alcohol intermediate which cyclizes at very high temperature ($\sim 160^\circ\text{C}$). Subsequent hydrolysis and Red-AlTM reduction afford the *N*-, 6-disubstituted isoquinuclidines.¹⁹

Advantageously, the synthetic route as depicted in Scheme 1 is employed in the present study. The Diels–Alder adduct was obtained in high yields using acrylonitrile as the dienophile to furnish nearly equal amounts of the *exo*- and *endo*-products (**9**) as shown by the NMR and UV intensity on TLC, the *exo* isomer being of slightly higher polarity. The reaction was also performed using allyl cyanide (vinylacetonitrile) as the dienophile, but furnished poor yields of the Diels–Alder adduct ($\sim 20\%$ in total) with a high *endo:exo* ratio (5:1). This approach was not optimized further.

Scheme 2 is followed in the synthesis of the analogs **11**–**15** from isoquinuclidine-6-carbonitrile (**10**). The amination of 4-chloroquinoline to give **11** proceeded in fair yield ($\sim 60\%$) with a reaction time of 48 h at high temperature (140°C). Lithium aluminum hydride reduction of the nitrile group to the amine gave excellent yields (90%), and dimethylation gave 50% yield. Ethylation with acetaldehyde produced the monosubstituted secondary amine **14** as the major product accompanied by some disubstituted product **15**. The products were purified by column chromatography.

Compound **6**, where the quinoline ring is attached to the isoquinuclidine through a methyleneamine bridge similar to that in chloroquine, was prepared from **10** as shown in Scheme 3. Methylation of **10** with formaldehyde and formic acid gave a 90% yield of **16**, which on lithium aluminum hydride reduction gave an 80% yield of **17**. Subsequent conversion of **17** to the target product **6** was achieved by the analogous procedure described in Scheme 2 for **11**–**15**.

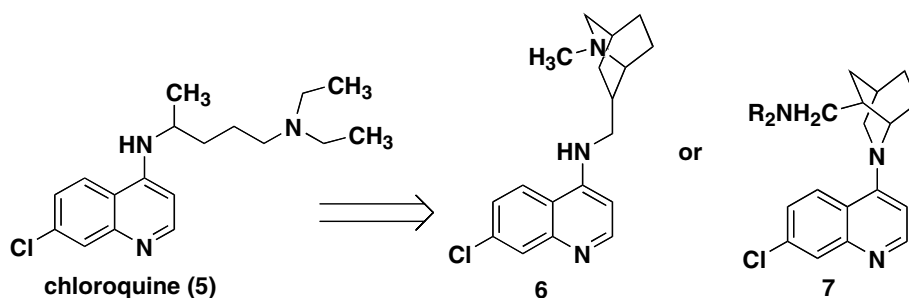
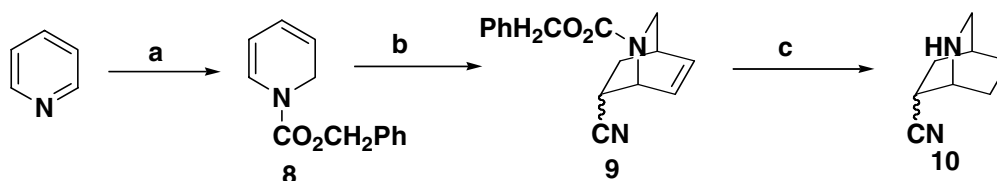
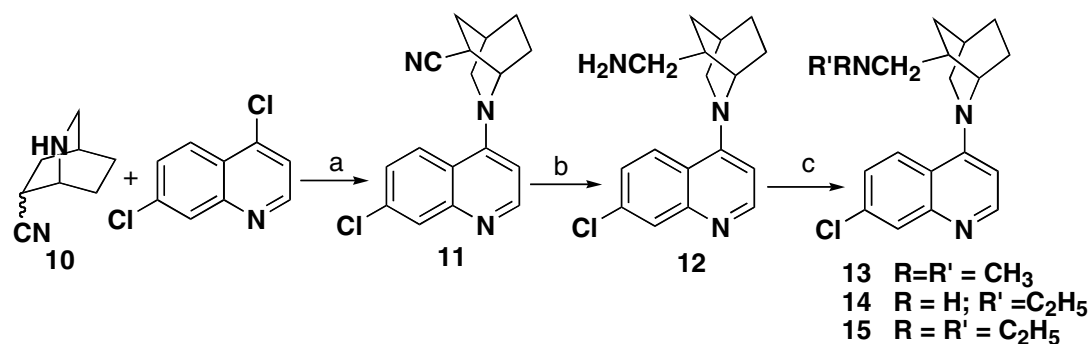


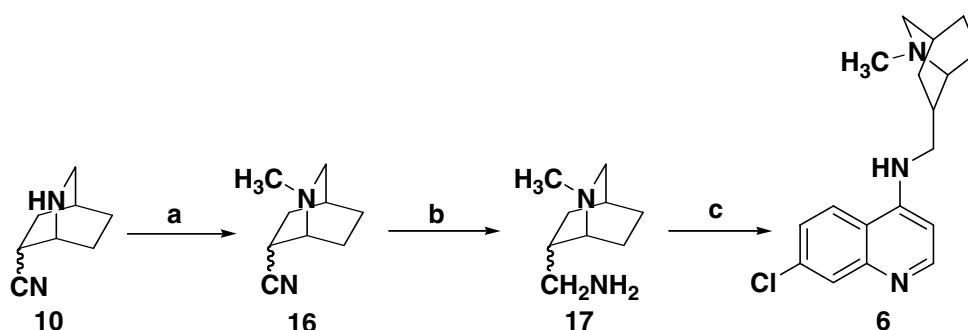
Figure 1.



Scheme 1. Reagents and conditions: (a) benzylchloroformate, NaBH_4 , -70°C , 3 h; (b) benzene, hydroquinone, acrylonitrile; (c) $\text{H}_2/\text{Pd-C}$, MeOH, overnight.



Scheme 2. Reagents and conditions: (a) K₂CO₃, DMF, 140 °C, 48 h; (b) LAH, rt, 18 h; (c) HCOOH, HCHO (13) or CH₃CHO (14, 15).



Scheme 3. Reagents and conditions: (a) HCOOH, HCHO, 60 °C, 8 h; (b) LAH, rt, 18 h; (c) 4,7-dichloroquinoline, DMF, K₂CO₃, 140 °C, 24 h.

3. Antimalarial activity

All the semirigid analogs of chloroquine synthesized in the present study (**6**, **11–15**) displayed significant in vitro antimalarial activity (Table 1). Compound **6** is the most potent of all these congeners being about 2-fold more potent against the chloroquine-sensitive D6 clone and about 30-fold more potent against the chloroquine-resistant W2 clone of *P. falciparum* than chloroquine. Its potency is similar to that of artemisinin against the D2 clone and 0.5-fold less potent against the W2 clone, which is also significant. Compound **6** possesses the same structural features as chloroquine, particularly the methyleneamine bridge. However, **6** has the semirigid side chain instead of the open alkylamine side chain of chloroquine. The isoquinuclidine N atom is substituted with the less bulky methyl group.

Compound **13** has the isoquinuclidine N attached to the quinoline ring, thus replacing the secondary amine of both chloroquine and **6** with a tertiary amine. In **13**, the terminal amine side chain is substituted with the less bulky methyl groups. Since it is as potent as chloroquine against the D2 clone and about 7-fold more potent against W2 clone, compound **13** is suggestive of a promising antimalarial drug.

Compounds **11**, **12**, **14** and **15** possess structural features similar to that of **13** except that they contain terminal nitrile, primary amine, ethylamine, and diethyl amine functions, respectively, furnishing relatively less potent agents. The reduced potency of nitrile-containing compound **11** may be due to the loss of the basic terminal amino group of chloroquine required for its antimalarial potency. Compounds **14** and **15** possess the more bulky

Table 1. In vitro antimalarial activity of isoquinuclidine analogs of chloroquine

Compound	Activity against <i>Plasmodium falciparum</i>				Cytotoxicity TC ₅₀ (ng/ml)
	<i>P. falciparum</i> . (D6 clone)		<i>P. falciparum</i> (W2 clone)		
	IC ₅₀ (ng/ml)	SI	IC ₅₀ (ng/ml)	SI	
6	3.8	>1252.3	4.0	>1190	NC
11	350	>13.6	170	>28	NC
12	130	>36.6	180	>26.4	NC
13	12	>396.7	18	>264.4	NC
14	280	>17	110	>43.2	NC
15	310	>15.4	200	>23.8	NC
Chloroquine	7.0	—	115	—	—
Artemisinin	3.6	—	1.8	—	—

NC, no cytotoxicity, selectivity index (SI) = IC₅₀ (Vero cells)/IC₅₀ (*P. falciparum*).

Table 2. In vitro antileishmanial activity of isoquinuclidine analogs of chloroquine

Compound	Activity against <i>Leishmania donovani</i>	
	IC ₅₀ (μg/ml)	IC ₉₀ (μg/ml)
6	3.0	6.5
11	16	33
12	4.2	20
13	1.9	6.0
14	7.2	29
15	3.8	8.0
Pentamidine	2.8	10
Amphotericin B	0.18	1.8

ethyl substituent, which might be responsible for its lower potency. Other stereochemical factors required for antimalarial potency have not been studied in great detail since a larger population of analogs would be required. However, the present study shows that exploration of rigid and semi-rigid congeners can lead to potentially useful increases in drug properties in this field. In general, it can be commented that as they possess similar structural features as chloroquine, they would be expected to act by a similar mechanism to chloroquine *viz.*, by interfering with the conversion of free heme to hemozoin. ^{20–23} The parasite thus accumulates large quantities of heme in the food vacuoles which lyses membranes, generates reactive oxygen intermediates, and inhibits many other processes important to the parasite. The *Plasmodium* parasites detoxify this heme by forming the hemozoin or malarial pigment. However, more detailed studies are needed to define the mode of action.

4. Antileishmanial activity

Most of these compounds also displayed potent in vitro antileishmanial activity (Table 2). Compound **11**, which contains a terminal nitrile function, is the least potent with **6** and **13** being the most potent (IC₅₀ 3.0 and 1.9 μg/ml, respectively). These potencies are comparable to that of the standard drug pentamidine (2.8 μg/ml), but are 10- to 40-fold less potent than amphotericin B (IC₅₀ 0.18 μg/ml). Few examples of antileishmanial activity of 4-amino- and 8-aminoquinoline derivatives have been reported, ^{24–26} and none possess any further detail about the mechanism of action.

5. Experimental

5.1. 1,2-Dihydropyridine-1-carboxylic acid benzyl ester (**8**)

Sodium borohydride (5.7 g, 0.15 mol) was added portionwise to a solution of anhydrous pyridine (12.1 ml, 0.15 mol) in anhydrous methanol (100 ml) at –70 °C under argon atmosphere. The reaction was cooled to –75 °C and benzylchloroformate (24.8 ml, 0.15 ml) added dropwise. The mixture was stirred for 3 h. Ether and water were added slowly to the reaction mixture that was then allowed to warm to room temperature.

The mixture was extracted with ether (3× 50 ml) and the combined ether layers washed with water (2× 40 ml) and then brine. The ether extract was dried with anhydrous MgSO₄ and evaporated to yield 31 g (96%) of **8** as a highly unstable oil which was used without further purification in the next step as described in the literature and was not characterized further. ²⁷

5.2. 7-Cyano-2-azabicyclo[2.2.2]oct-5-ene-2-carboxylic acid benzyl ester (**9**)

The dihydropyridine derivative **8** was dissolved in benzene (50 ml), and 40 ml of acrylonitrile and 300 mg hydroquinone added. The mixture was kept at room temperature for 5 h, then heated at 80 °C for 3 days. An additional 40 ml of acrylonitrile was added and heating under reflux continued for an additional 2 days. The reaction mixture was cooled to room temperature, evaporated to dryness, and the residue purified by column chromatography over silica gel (200–425 mesh) and eluted with hexane/ethyl acetate (80:20) to yield 15 g of a mixture of the *exo* and *endo* adducts as a colorless oil that solidified on standing. A portion was purified to separate *exo*- and *endo*-isomers for analytical purposes.

¹H NMR (400 MHz; CDCl₃): *exo*: 7.35 (5H, m, Ar), 6.35–6.5 (2H, m, 7,8-ethylene), 5.15 (2H, m, CH₂-Bz), 4.9–5.1, (1H, m, CH-1), 3.1–3.5, (2H, m, CH₂-3), 2.6–2.85 (2H, m, 2× CH-4 & 6), 1.85 (2H, m, CH₂-5). *endo*: 7.35 (5H, m, Ar), 6.4–6.6 (2H, m, 7,8-ethylene), 5.1 (2H, m, CH₂-Bz), 4.9–5.3, (1H, m, CH-1), 2.8–3.25 (4H, m, 2× CH-4 & 6, CH₂-3), 1.6–2.0 (2H, m, CH₂-5). (EIMS: 269.4 M+1) 270.3 (M+2).

5.3. 2-Azabicyclo[2.2.2]octane-6-carbonitrile (**10**)

To 5 g of the Diels–Alder adduct **9** (*endo*) dissolved in methanol was carefully added 500 mg of Pd–C (5% w/w). The mixture was hydrogenated overnight at 60 psi, and then filtered under vacuum through a short column packed with silica at the bottom and Celite on the top. The filtrate was evaporated and the product left under high vacuum for 1 h to yield 2.7 g (99%) of **10**. The product was stored under argon atmosphere and used immediately for the next reaction.

¹H NMR (400 MHz; CDCl₃): 2.6–3.1 (2H, m, 2× CH-1 & 6), 1.4–2.0 (5H, m, CH-4, 2× CH₂-3,5), 1.0–1.2 (4H, m, CH₂-7 & 8). EIMS: 137.2 (M+1).

5.4. 2-(7-Chloro-quinolin-4-yl)-2-aza-bicyclo[2.2.2]octane-6-carbonitrile (**11**)

Compound **10** (2.5 g) was added to a hot mixture of DMF (4 ml), 4,7-dichloroquinoline (400 mg), and K₂CO₃ (600 mg). The mixture was heated to 140 °C for 48 h, evaporated to dryness, and the residue partitioned between water (50 ml) and dichloromethane (50 ml). The dichloromethane layer was separated and the aqueous layer extracted twice more with dichloromethane. The combined dichloromethane extracts were washed with water and brine, dried over anhydrous

MgSO₄, and evaporated to yield the crude product. Column chromatography over silica gel (200–425 mesh) using chloroform/methanol (9:1) yielded 3.9 g of product which was used without further purification for the next step. For analytical purposes a portion of this was purified by column chromatography over silica gel (200–425 mesh) using dichloromethane/methanol (9:1) as solvent.

¹H NMR (400 MHz; CDCl₃): 8.4 (1H, m, Ar), 7.9 (2H, m, Ar), 7.3 (1H, m, Ar), 6.3 (1H, m, Ar), 3.3–4.2 (3H, m, CH-1, CH₂-3), 1.3–2.5 (8H, m, 2× CH-4 & 6, 3× CH₂-5,7,8). EIMS: 298.3 (M+1).

5.5. [N-(7-Chloro-quinolin-4-yl)]-6-aminomethyl-2-azabicyclo[2.2.2]octane (12)

To 100 mg (0.34 mmol) of the nitrile **11** in dry THF (1 ml) was added drop wise 500 µl of 1 M lithium aluminum hydride (LAH) solution in ether under argon atmosphere at 0 °C. The reaction mixture was stirred for 18 h at room temperature, and then quenched by the dropwise addition of 0.5 ml of water at 0 °C. The reaction mixture was extracted with ether (3× 5 ml) and the aqueous precipitate extracted once again with 10 ml of ether under reflux. The combined organic extracts were washed with brine, dried with anhydrous MgSO₄, and evaporated to yield the crude product which was used without further purification for the next step. An analytical grade sample was obtained from a portion of this by column chromatography over silica gel (200–425 mesh) using dichloromethane/methanol/ammonia (9:0.9:0.1) as solvent.

¹H NMR (400 MHz; CDCl₃): 8.4 (1H, m, Ar), 7.65–8.0 (2H, m, Ar), 7.3 (1H, m, Ar), 6.3 (1H, m, Ar), 3.0–3.2 (3H, m, CH-1, CH₂-3) 1.3–2.7 (12H, m, 2× CH-4 & 6, 3× CH₂-5,7,8, CH₂-NH₂). EIMS: 302.3 (M+1), 303.3 (M+2), 304.3 (M+3).

5.6. [2-(7-Chloro-quinolin-4-yl)]-6-dimethylaminomethyl-2-azabicyclo[2.2.2]octane (13)

The amine was placed in a 25 ml round bottom flask and to this were added 100 µl of 37% formaldehyde solution and 200 µl of formic acid. The mixture was heated under reflux at 70 °C for 20 h. The reaction mixture was cooled, and 5% NaOH added until alkaline whereupon it was extracted with chloroform, dried over anhydrous MgSO₄ and evaporated to dryness to provide the crude product. This product was then chromatographed over silica gel (200–425 mesh), eluting with chloroform/methanol (8:2) to wash out the less polar impurities, and then with chloroform/methanol/ammonia (8:1.75:0.25) to provide 30 mg of pure **13**.

¹H NMR (400 MHz; CDCl₃): 8.4 (1H, m, Ar), 7.9–7.7 (2H, m, Ar), 7.3 (1H, m, Ar), 6.3 (1H, m, Ar), 3.0–3.3 (3H, m, CH-1, CH₂-3), 2.2 (6H, s, N(CH₃)₂), 1.9–2.3 (2H, m, overlapped with CH₃ peaks, CH₂-N), 1.3–1.85 (8H, m, 2× CH-4 & 6, 3× CH₂-5,7,8). EIMS: 332.3 (M+3).

Using acetaldehyde rather than formaldehyde in the above method yielded **14** and **15**.

¹H NMR (400 MHz; CDCl₃) (**14**): 8.4 (1H, m, Ar), 8.0 (1H, m, Ar), 7.9, (1H, m, Ar), 7.3 (1H, m, Ar), 6.3 (1H, m, Ar), 3.0–3.3 (3H, m, CH-1, CH₂-3), 1.2–2.3 (12H, m, 2× CH, 5× CH₂), 0.9 (3H, t, CH₃). EIMS: 332.3 (M+3).

¹H NMR (400 MHz; CDCl₃) (**15**): 8.4 (1H, m, Ar), 7.7–7.95 (2H, m, Ar), 7.3 (1H, m, Ar), 6.3 (1H, m, Ar), 3.0–3.3 (3H, m, CH-1, CH₂-3), 0.9–2.6 (20H, m, 2× CH, 6× CH₂, 2× CH₃). EIMS: 360.3 (M+3).

5.7. 2-Methyl-2-azabicyclo[2.2.2]octane-6-carbonitrile (16)

To 880 mg of **10** was added 900 µl of 37% formaldehyde in water with cooling. Then 1.5 ml of formic acid (95%) was added dropwise with cooling, the resulting mixture stirred for an hour at room temperature, and refluxed for 6 h. The reaction mixture was cooled, 5% NaOH solution added to raise the pH >11, and extracted with dichloromethane (3× 10 ml). The organic layers were combined, washed with brine, dried with MgSO₄, and evaporated to yield **16** (870 mg, 90%). A portion was purified for analytical purposes and the remainder used without further purification in the next step.

¹H NMR (400 MHz; CDCl₃): 3.1 (1H, m, CH-1), 2.6–2.75 (2H, m, CH₂-3), 2.35 (3H, s, N-CH₃), 1.85–2.25 (4H, m, 2× CH-4 & 6, CH₂-3), 1.3–1.75 (4H, m, 2× CH₂-7 & 8). EIMS: 151.2 (M+1).

5.8. 6-Aminomethyl-2-methyl-2-azabicyclo[2.2.2]octane (17)

To 750 mg (5 mmol) of **16** in 5 ml of anhydrous THF was added drop wise 5 ml of 1 M solution of LAH in ether at 0 °C. The mixture was stirred at room temperature for 20 h, cooled to 0 °C, and then a mixture of 1 ml of water and 10 ml of ether was carefully added. The mixture was stirred overnight and the organic layer taken off by decantation, washed with brine, and dried with anhydrous MgSO₄. Evaporation of solvent to afford a yield of 600 mg (80%) of **17** which was used without further purification in the next step.

5.9. (7-Chloro-quinolin-4-yl)-(2-methyl-2-azabicyclo[2.2.2]oct-6-ylmethyl)amine (6)

To a hot mixture of DMF (1 ml), 4,7-dichloroquinoline (400 mg), and K₂CO₃ (600 mg) was added 300 mg of **17**. The mixture was heated to 140 °C for 24 h, evaporated to dryness, and then treated with water (10 ml) and dichloromethane (10 ml). The dichloromethane layer was separated and the aqueous layer extracted twice more with dichloromethane. The combined dichloromethane extracts were washed with water and brine, dried with anhydrous MgSO₄, and evaporated to yield the crude product which was purified by column chromatography over silica gel (200–425 mesh) using chloroform/methanol (9:1) to yield 300 mg of **6**.

^1H NMR (400 MHz; CDCl_3): 9.2 (1 H, br s, NH-Ar), 8.4 (1H, m, Ar), 7.9 (1H, m, Ar), 7.65 (1H, m, Ar), 7.3 (1H, m, Ar), 6.3 (1H, m, Ar), 3.2–3.5 (3H, m, CH-1, $\text{CH}_2\text{-N-Ar}$), 2.7 (1H, m, CH-6), 2.4 (3H, s, N- CH_3), 1.4–2.3 (9H, m, CH-4, $4\times \text{CH}_2\text{-3,5,7,8}$). EIMS: 316.3 (M+1).

5.10. Bioassays

5.10.1. In vitro antimalarial assay. The compounds were assayed for in vitro antimalarial activity against the W2 clone and D6 clone of the pathogenic *Plasmodium falciparum*. The strains of *P. falciparum* were obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC. Strains of Sierra Leon D6 and Indochina W2 are chloroquine-sensitive and chloroquine-resistant, respectively. The parasite was grown in type A human RBCs and, two strains subcultured daily with fresh medium and blood cells. On the day of the assay, a suspension of infected blood cells (2% parasitemia and 2% hematocrit) was prepared using type A human red blood cells. Assays were performed by standard 96-well flat-bottomed microplate methods and done in duplicate at different concentrations. IC_{50} values were obtained from the dose curves. Artemisinin and chloroquine were included in each assay as the drug controls and DMSO was used as vehicle control. The whole protocol was developed based on the published method of Makler.²⁸ The cytotoxicity assays were also performed in parallel against the Vero cells and the selectivity index was measured. A selectivity index of >9 was considered to indicate a lead for further development of antimalarial drug.

5.10.2. In vitro antileishmanial assay. Antileishmanial activity was tested in vitro on a culture of *Leishmania donovani* promastigotes. In a 96-well microplate assay compounds with appropriate dilution in DMSO (5 μL) were added to the leishmania promastigote culture (2×10^6 cells/mL). The plates were incubated at 26°C for 72 h and growth of leishmania promastigotes was determined by the Alamar blue assay,²⁹ modified to a fluorometric assay. Pentamidine and Amphotericin B were used as the standard antileishmanial agents. All the analogs were simultaneously tested for cytotoxicity on VERO (monkey kidney fibroblast) cells using the Neutral Red assay.³⁰ The IC_{50} value for each compound was computed from the growth inhibition curve.

6. Conclusion and future directions

This study introduces a new class of chloroquine analogs by replacing the alkylamino side chain with the bicyclic amine, isoquinuclidine, which provides improved in vitro activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* as well as *L. donovani*, the causative agent of fatal visceral leishmaniasis. Future studies will be directed toward determination of the effect of stereochemistry on activity utilizing stereospecific synthetic methods of isoquinuclidines.^{31–34} The in vivo activity

of the compounds will also be studied in addition to their activity on hemozoin formation²⁰ to demonstrate the mechanism of their antimalarial activity.

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References and notes

- Robertson, G. B.; Tooptakong, U.; Lamberton, J. A.; Geewanda, Y. A.; Gunawardana, P.; Bick, I. R. C. *Tetrahedron Lett.* **1984**, 25, 2695.
- Page, C. B.; Pinder, A. R. *J. Chem. Soc.* **1964**, 4811.
- Broadbent, J. L.; Schnieden, H. *Br. J. Pharmacol.* **1958**, 13, 213.
- Nagata, K.; Aistrup, G. L.; Honda, H.; Shono, T.; Narahashi, T. *Pestic. Biochem. Physiol.* **1999**, 64, 157.
- Aube-Lecomte *Arch. Med. Navale* **1864**, 2, 264.
- Dyvovsky, J.; Landrin, E. C. *R. Acad. Sci. (Paris)* **1901**, 133, 748.
- Landrin, A. *Bull. Sci. Pharmacol.* **1905**, 11, 319.
- Taylor, W. I. *J. Am. Chem. Soc.* **1957**, 79, 3298.
- Buchi, G.; Coffen, D. L.; Koksik, K.; Sonnet, P. E.; Ziegler, F. E. *J. Am. Chem. Soc.* **1966**, 88, 3099.
- Popik, P.; Layer, R. T.; Skolnick, P. *Pharmacol. Rev.* **1995**, 47, 235.
- Popik, P.; Glick, S. D. *Drug Future* **1996**, 21, 1109.
- Levi, M. S.; Borne, R. F. *Curr. Med. Chem.* **2002**, 9, 1807.
- Sundberg, R. J.; Smith, S. Q. *Alkaloids* **2002**, 59, 281.
- Kuhne, M. E.; He, L.; Jokiel, P. A.; Pace, C. J.; Fleck, M. W.; Maisonneuve, I. M.; Glick, S. D.; Bidlack, J. M. *J. Med. Chem.* **2003**, 46, 2716.
- Khan, M. O. F.; Levi, M. S.; Clark, C. R.; Ablordeppey, S. Y.; Law S.-J.; Wilson, N. H.; Borne, R. F. In *Studies in Natural Product Chemistry*, Rahman, Ata-Ur, Ed.; Elsevier Science: Amsterdam, **2007**, in press.
- Hyde, J. E. *Trend Parasitol.* **2005**, 21, 494.
- Crouse, J. R.; Pinder, A. R. *J. Nat. Prod.* **1989**, 52, 1227.
- Borne, R. F.; Clark, C. R.; Peden, R. L. *J. Heterocycl. Chem.* **1973**, 10, 241.
- Law, S. J.; Borne, R. F. *Eur. J. Med. Chem.* **1980**, 15, 229.
- Krogstad, D. J.; Schlesinger, P. H.; Gluzman, I. Y. *Parasitol. Today* **1992**, 8, 183.
- Sullivan, David J., Jr.; Gluzman, I. Y.; Russell, D. G.; Goldberg, D. E. *Proc. Natl. Acad. Sci.* **1996**, 93, 11865.
- Meshnick, S. R. *Ann. Trop. Med. Parasitol.* **1996**, 90, 367.
- Asawamahsakda, W.; Ittarat, I.; Chang, C.-C.; McElroy, P.; Meshnick, S. R. *Mol. Biochem. Parasitol.* **1994**, 67, 183.
- Sahu, N. P.; Pal, C.; Mandal, N. B.; Banerjee, S.; Raha, M.; Kundu, A. P.; Basu, A.; Ghosh, M.; Roy, K.; Bandyopadhyay, S. *Bioorg. Med. Chem.* **2002**, 10, 1687.
- Fournet, A.; Angelo B. A.; Munoz, V.; Hocquemiller, R.; Roblot, F.; Bruneton, J.; Richomme, P.; Gantier, J. C. *PCT Int. Appl.* 1993, WO9307125A1.
- Berman, J. D.; Lee, L. S. *Am. J. Trop. Med. Hyg.* **1983**, 32, 753.

27. Street, L. J.; Baker, R.; Book, T.; Kneen, C. O.; McLeod, A. M.; Merchant, K. J.; Showell, G. A.; Saunders, J.; Herbert, R. H.; Freedman, S. B.; Harley, E. A. *J. Med. Chem.* **1990**, *33*, 2690.
28. Makler, M. T.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205.
29. Mikus, J.; Steverding, D. *Parasitol. Int.* **2000**, *48*, 265.
30. Babich, H.; Borenfreund, E. *Appl. Env. Microbiol.* **1991**, *57*, 2101.
31. Dos Santos, D. C.; Gil, R. P. deF.; Gil, L.; Marazano, C. *Tetrahedron Lett.* **2001**, *42*, 6109.
32. Gil, L.; Gateau, O. A.; Marazano, C.; Das, B. C. *Tetrahedron Lett.* **1995**, *36*, 707.
33. Krow, G. R.; Carey, J. T.; Cannon, K. C.; Henz, K. J. *Tetrahedron Lett.* **1982**, *23*, 2527.
34. Marazano, C.; Yannic, S.; Genisson, Y.; Mehmandoust, M. B.; Das, B. C. *Tetrahedron Lett.* **1990**, *31*, 1995.